

REMARKS

Reconsideration of the above-identified application in view of the amendments above and the remarks following is respectfully requested.

Claims 20-38 are in this case. Claims 22-26 and 35-38 were withdrawn from further consideration by the Examiner, under 37 C.F.R. § 1.142(b) as being drawn to a non-elected species. Claims 20-21 and 27-34 have been rejected. Claims 20-21 and 27-34 have now been amended. New claims 39-64 have now been added, being supported by the original specification and thereby not constituting new subject matter.

35 U.S.C. § 112, Second Paragraph, Rejections

The Examiner has rejected claims 20-21 and 27-34 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. The Examiner's rejections are respectfully traversed. Claims 20, 30 and 32-34 have now been amended. Claims 39, 41, 44-51, 58, and 66, and 62-64 have now been added.

The Examiner contends that the recitation "HLA-compatible, allogeneic peripheral blood lymphocytes" is vague and indefinite on the basis of the contention that the term "HLA-compatible" is not defined in the specification, whereas the disclosure on page 11, includes some discussion of the types of stem cells that might be acceptable for transplantation, the "HLA-compatible" cells of the claims are not actually defined. The Examiner further states that the additional limitations of claims 32-34 cannot serve to define the cells on the basis of the limitations not disclosing which cells are encompassed and which are not, in the absence of the limitations.

Applicant wishes to first respectfully point out that the concept of "HLA-compatibility" was thoroughly standardized and the use of such terminology widely employed in the art at the time the instant invention was made (refer, for example, to enclosed abstracts of: Beatty, 1994; Buckley *et al.*, 1993; and Henslee-Downey PJ., 1993). As such, Applicant is of the strong opinion that the term "HLA-compatible" does not require explicit definition in the specification so as to be clearly and unambiguously understood by the ordinarily skilled artisan.

Applicant wishes to further respectfully point out that, in very sharp contrast

to the Examiner's assertion, the instant specification indeed nevertheless clearly and unambiguously defines HLA compatibility between the donors and the recipients of the instant invention (and hence HLA compatibility between a donor/donor tissues/donor cells and a recipient/recipient tissues/recipient cells), according to universally employed, standard art terminology. Namely, the specification clearly and unambiguously defines a donor/donor cells/donor tissues and a recipient/recipient tissues/recipient cells which are "HLA-compatible" according to universal/standard art terminology as being at least haploidentical (specification, second paragraph of page 11), or fully haplotype-matched or single HLA locus mismatched (specification, second paragraph of page 11; and second paragraph of page 20).

Applicant further wishes to yet further respectfully point out that the description of autologous stem cells on page 11 is drawn to the tissue sources from which such cells may be obtained and that the scope of such description is indeed met in the term "peripheral blood" of claim 20 with respect to the allogeneic lymphocytes of the instant invention. However, the Examiner appears to be objecting to the term "HLA-compatible" which is a genotype specific parameter and not a differentiation lineage specific parameter, to which the stem cell description on page 11 is drawn. Hence, in Applicant's opinion, the analogy drawn between such genotype specific parameter and differentiation lineage specific parameter is inapplicable and hence does not provide a basis for this rejection.

Nevertheless, in the interest of further clarifying the claims language in this respect, and in the interest of expediting prosecution of the instant application, Applicant presently elects to make the following amendments:

(i) claim 20 is now amended so as to replace the limitation of administered lymphocytes being HLA-compatible, with that of administered lymphocytes being derived from a allogeneic donor allogeneic with the patient;

(ii) claim 32, depending from claim 20, is now amended so as to replace the limitation of HLA-compatible cells being fully HLA-matched with the patient with that of the lymphocyte donor being fully HLA-matched with the recipient;

(iii) claim 33, depending from claim 20, is now amended so as to replace the limitation of HLA-compatible cells being at least haploidentical with the patient with that of the lymphocyte donor being at least HLA-haploidentical with the patient;

(iv) claim 34, depending from claim 20, is now amended so as to replace the limitation of HLA-compatible cells being single HLA locus mismatched cells from a sibling of the patient, with that of the lymphocyte donor being HLA haplotype-mismatched at a single HLA locus with the patient; and

(v) new claim 39, depending from claim 20, has been added including the limitation of the lymphocyte donor being a sibling of the patient.

Support for language employed in the above described amendments is provided, in accordance with the argumentation above, in the specification, second paragraph of page 11 and second paragraph of page 20. In particular, support for amending claim 20 to include the limitation of administered lymphocytes being derived from a lymphocyte donor allogeneic with the patient is provided in the specification on page 20, second paragraph, first sentence.

The limitation of the administered lymphocytes being PBLs removed from currently amended claim 1 has been included in new claim 58 depending from claim 20.

The Examiner contends that the recitation “administering peripheral blood lymphocytes in a regimen that causes a clinically mild graft-versus-host (GVH) response,” is vague and indefinite on the basis of the Examiner’s contention that the metes and bounds of said “clinically mild GVH response” are not defined in the specification.

Applicant firstly wishes to respectfully point out that above and beyond the widely and clearly understood meaning in the art of the term “clinically mild GVH responses,” as further clarified hereinbelow, the instant specification nevertheless indeed quite specifically and explicitly defines the meets and bounds of said “clinically mild GVH response”, in very sharp contrast to the Examiner’s assertion. Namely, the specification specifically and explicitly characterizes grade I/II GVH responses as corresponding to minimal GVH responses (specification, page 38, sentence starting on line 16 to end of paragraph). Such “minimal” GVH responses will be clearly understood by the ordinarily skilled artisan as being the same as “mild” GVH responses. Therefore, Applicant is of the very strong opinion that the metes and bounds of said “clinically mild GVH responses” are indeed very adequately defined in the specification.

Applicant wishes to further respectfully point out that, at the time the instant invention was made, "mild GVH responses" were routinely diagnosed by the ordinarily skilled artisan as GVH responses which were detectable but which either did not require medical intervention to prevent unacceptable pathogenesis in the patient (refer, for example, to enclosed abstracts of: Kohler *et al.*, 1988; and Bassukas, 1992), or as GVH responses which could be prevented from causing unacceptable pathogenesis via pharmacological treatment.

The Examiner further contends that the instant specification discloses on page 8 that GVH responses include both classic clinical symptoms thereof as well as molecular/cellular responses correlating therewith, but does not adequately define such molecular/cellular responses encompassed by the method of the claims.

Applicant wishes to respectfully point that, in accordance with the clarifications set forth above, the clinically mild GVH responses referred to in claim 20 are indeed precisely defined in the specification, both with respect to standardized grade as well as with respect to anatomic involvement. Furthermore, it is Applicant's strong opinion that at the time the instant invention was made, the measurement of numerous molecular and cellular responses for detecting, characterizing and predicting such defined GVH responses was routinely performed in the art (refer, for example, to enclosed abstracts of: Dickinson AM. *et al.*, 1994; Guiot *et al.*, 1987; Hymes *et al.*, 1985; Renkonen and Hayry, 1987; Rowbottom *et al.*, 1993; Sahmoud *et al.*, 1993; Vogelsang GB. *et al.*, 1985; and Weisdorf *et al.*, 1983). Indeed, such standard criteria for grading GVH responses were in use 20 years prior to time the instant invention was made (refer, for example, to enclosed abstract of Przepiorka *et al.*, 1994). As such, in Applicant's very strong opinion, by virtue of corresponding to precisely defined GVH responses which were known in the art to correspond to specific molecular/cellular responses, the molecular/cellular responses encompassed by the method of the claims will be known to one ordinarily versed in the art, and, as such, are indeed clearly defined by the instant specification, in sharp contrast to the Examiner's assertion.

Nevertheless, in the interests of further clarifying the claims language, and in the interest of expediting prosecution of the instant application, Applicant currently elects to:

(i) amend claim 20 so as to replace the limitation of administering lymphocytes in a regimen that causes a clinically mild GVH response, with the limitation of administering lymphocytes in a regimen selected so as to cause at least partial engraftment of the lymphocytes;

(ii) add new claim 41 depending from claim 20 including the limitation of said regimen selected so as to cause at least partial engraftment of the lymphocytes being selected so as to cause full engraftment of the lymphocytes; and

(iii) amend claim 20 so as to include the step of administering a dose of allogeneic stem cells in a regimen selected so as to cause minimal GVHD.

Support for amending claim 20 so as to include such limitations using such amendment language and for adding new claim 41 using the language employed is provided in the specification, pages 37-38, section entitled "Patient No. 8". In order to yet further clarify the claims language, and in the further interest of expediting issuance of the instant application, Applicant presently elects to add new claims 44-51 depending on claim 20 which respectively include the limitations of said minimal GVHD being: GVHD having a grade selected from a range of grade I to grade II; mucocutaneous GVHD; GVHD involving the oral cavity; GVHD involving the skin; GVHD not substantially involving the intestines; GVHD not substantially involving the skin; acute GVHD; and chronic GVHD. Support for addition of new claims 44-51 using such claims language is provided in the specification, pages 37-30, section entitled "Patient No. 8", in particular sentence starting on page 38, line 16 to end of paragraph; and page 50, paragraph entitled "Patient No. 8". Support for addition of new claims 40-46 using such claims language, and for amending the text to include the above paragraph, is provided in the specification, pages 37-30, section entitled "Patient No. 8", in particular sentence starting on page 38, line 16 to end of paragraph; and page 50, paragraph entitled "Patient No. 8".

The Examiner contends that the recitation "wherein said administering is after the patient is partially hematopoiesis recovered but is not fully immune reconstituted," is vague and indefinite as the metes and bounds of the time frame encompassed by this limitation are not defined in the specification.

Applicant wishes to point out that it will be clear to the ordinarily skilled artisan that a human cancer patient having undergone a malignant cell debulking

procedure requiring incident stem cell transplantation, as extensively discussed and described throughout the specification, in particular in Example 2, will be a human cancer patient having undergone a debulking procedure leading to loss of hematopoiesis in the patient. It will be furthermore evident to one of ordinary skill in the art that such a patient following such stem cell transplantation will generally undergo a progressive hematopoietic recovery as a result of such stem cell transplantation. It will be additionally clear to ordinarily skilled artisan that such a patient having undergone such stem cell transplantation for hematopoietic recovery

from such hematopoietic loss will inherently not be fully immune reconstituted prior to full hematopoietic recovery. As such, it will be understood by one of ordinary skill in the art that administration of lymphocytes to a patient which is partially hematopoietically recovered in such a context will inherently be administration of lymphocytes to a patient which is not fully immune reconstituted. Therefore, it will be obvious to one of ordinary skill in the art that the metes and bounds of the time frame encompassed by the limitation “wherein said administering is after the patient is partially hematopoiesis recovered but is not fully immune reconstituted,” clearly includes the period delimited by the time of stem cell transplantation to the time of full hematopoietic recovery. Since, the specification clearly indicates that the method must be performed by continuous monitoring of hematopoietic recovery, and since the specification provides very clear guidelines as to how such monitoring must be performed (refer, for example, to: page 18, sentence starting on line 21; and Example 2, sections relating to Patient No’s. 5, 7, 8, 10, 12, 17, and 18, specifically in relation to stabilization of blood cell counts). As such, in Applicant’s strong opinion, the metes and bounds of the time frame encompassed by the “wherein said administering is after the patient is partially hematopoiesis recovered but is not fully immune reconstituted,” are indeed clearly defined in the specification.

Nevertheless, in the interest of clarifying the claims language, and in the interest of expediting issuance of the instant application, Applicant presently elects to: (i) amend claim 20 to include the limitation of said malignant cell debulking procedure being associated with at least partial loss of hematopoiesis; (ii) amend claim 20 so as to omit the limitation of administering said peripheral blood lymphocytes to the patient after the patient is partially hematopoiesis recovered but is

not fully immune-reconstituted; (iii) add new claim 61, depending from claim 20, including the limitation of administering lymphocytes to the patient following said at least partial engraftment of said lymphocytes in the patient; (iv) add new claim 60, depending from claim 20, including the limitation of administering lymphocytes to the patient during a period selected from the range of 90 to 124 days following said autologous stem cell transplantation; (v) add the new method step of administering allogeneic stem cells in a regimen selected so as to cause minimal GVHD; (vi) add new claim 62, depending from claim 20, including the limitation of administering, following administration of lymphocytes, allogeneic stem cells in a regimen selected so as to cause minimal GVHD; (vii) add new claim 63, depending from claim 20, including the limitation of administering allogeneic stem cells in a regimen selected so as to cause minimal GVHD following at least partial engraftment of previously administered lymphocytes; and (viii) add new claim 64, depending from claim 20, including the limitation of monitoring the patient for levels of malignant cells deriving from said population prior to administration of allogeneic stem cells. Support for these amendments and new claims, and the language thereof, is provided in the specification, pages 37-38, section entitled "Patient No. 8".

The phrase "where in," of claim 30 has now been amended to properly recite "wherein," in accordance with the Examiner's remarks.

In view of the above arguments and amendments, Applicant believes to have overcome the 35 U.S.C. § 112, second paragraph, rejections.

35 U.S.C. § 103(a) Rejections - Slavin (1992) in view of Johnson et al. (1993) and Slavin et al. (1990).

The Examiner has rejected claims 20-21, 27-28, 30 and 32-34 under 35 U.S.C. § 103(a) as being unpatentable over Slavin (1992) in view of Johnson et al. (1993) and Slavin et al. (1990). The Examiner's rejections are respectfully traversed. Claim 20 has now been amended. New claims 40, 42-51, 52-57, 60-61 and 63-64 have now been added.

The Examiner contends that Slavin teaches the basic concept of the invention of the instant claims, i.e., autologous stem cell transplantation after tumor debulking followed by an infusion of allogeneic donor lymphocytes intended to achieve a GVL

effect, which may include GVHD, as a treatment for a hematologic malignant disease such as leukemia. The Examiner further contends that the reference teaching differs from the claimed invention only in that it does not teach some of the specific limitations of the claims, such as the use of HLA-compatible donor lymphocytes, the induction of a mild GVH response, the timing of therapy, i.e., the administration of donor lymphocytes after partial hematopoietic recovery, the source of said lymphocytes (bone marrow or peripheral circulation), or the type of cancer (leukemia or lymphoma).

The Examiner still further contends that Johnson *et al.* teaches that, in the absence of GVHD, the rate of leukemia relapse is greater because it is donor T-cells that are responsible for the GVL effect, and contends that the reference discusses the possibility of inducing GVL while minimizing GVHD and teaches that insignificant or mild GVHD is acceptable as part of the GVL effect.

The Examiner yet further contends that Slavin *et al.* teaches administration of donor lymphocytes after partial hematopoietic recovery and the use of HLA-compatible lymphocytes.

The Examiner contends that it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to perform autologous stem cell transplantation after tumor debulking followed by an infusion of allogeneic donor lymphocytes intended to achieve a GVL effect which may include GVHD as a treatment for a malignant hematopoietic malignant disease, such as leukemia, as taught by Slavin. The Examiner concludes that one of ordinary skill in the art at the time the invention was made would have been motivated to allow a certain degree of GVHD, as taught by Johnson *et al.*, because this would have been a minor trade-off for effective GVL, i.e., the T-cells that induce GVL also induce some GVHD, and would have been motivated to administer the donor lymphocytes after partial hematopoietic recovery and the use of HLA-compatible lymphocytes, as taught by Slavin *et al.*, because this timing of administration would seem logical. The Examiner states that administering the donor lymphocytes with the bone marrow was well known to induce GVHD while administering the donor lymphocytes long after transplantation would allow for the residual disease to reestablish itself. The Examiner states that the "monitoring" requirement of the claims comprises a routine

part of all oncological treatments and is therefore obvious. The Examiner states that claims 27 and 28 are included in the rejection because bone marrow and peripheral circulation are the most obvious sources of human stem cells, and that claims 32-34 are included in the rejection because the claims recite the types of donor cells that would most obviously be "HLA-compatible", i.e., fully HLA-matched, haploidentical, and single HLA locus-mismatched. The Examiner states that it is well known in the transplantation arts to seek the most compatible donors for successful transplantation and that the recitation of such would be obvious.

Applicant vigorously disagrees with the Examiner's contention that Slavin *et al.* teaches administration of donor lymphocytes after substantial partial hematopoietic recovery, and hence that one of ordinary skill in the art at the time the invention was made would have been motivated to administer the donor lymphocytes after substantial partial hematopoietic recovery in light of Slavin *et al.* Applicant wishes to point out that in fact Slavin *et al.* teaches administration of donor lymphocytes on the day following ASCT which would certainly not be considered by the ordinarily skilled artisan at the time the claimed invention was made to be sufficient time to enable substantial partial hematopoietic recovery. In sharp contrast, the instant application teaches for the first time administration of donor lymphocytes more than one day following ASCT, after one of ordinary skill in the art would concur that partial hematopoietic recovery has indeed occurred [specification, Example 2: Patient No. 18, day +35 (week +5); Patient No. 12, day +42 (week +6); Patient No. 16, day +49 (week +7); Patient No. 7, day +55; Patients Nos. 9 and 17, day +56 (week +8); Patient No. 6, day +58; Patients Nos. 10-11, day +70 (week +10); Patient No. 5, day +71; Patient No. 13, day +77 (week +11); Patient No. 8, day +90; Patient No. 14, day +98 (week +14); and Patient No. 14, day +140 (week +20)].

Applicant wishes to respectfully point out that, in sharp contrast to the Examiner's contention, the state-of-the-art at the time the invention was made was such that one of ordinary skill in the art would instead have thought it important to administer the donor lymphocytes prior to any substantial hematopoietic recovery in order to avert their rejection by newly recovered host lymphocytes derived from the autologous bone marrow graft. Applicant wishes to particularly point out that it was the results disclosed for the first time in the instant application which indicated that it

was possible and/or preferable to wait for substantial partial hematopoietic recovery prior to administering the donor lymphocytes. The instant specification teaches for the first time that it is desirable to wait for at least substantial partial recovery since GVHD due to the donor lymphocytes would be less severe, and less potentially lethal, than if the donor lymphocytes were given before any substantial recovery (specification, page 18, lines 10-14). The instant specification also teaches for the first time that it is possible to wait for substantial partial recovery before administering the donor lymphocytes since only transient chimerism is required for therapeutic benefit (specification, page 18, lines 3-10), and that, furthermore, such transient chimerism would also have the benefit of decreasing the incidence and severity of GVHD mediated by the donor lymphocytes. Thus, the instant specification teaches for the first time the safe administration of fully activated HLA-mismatched lymphocytes while avoiding the risk of uncontrolled GVHD.

Applicant moreover wishes to respectfully point out that Johnson *et al.* in fact suggests transplantation of allogeneic donor derived stem cells prior to administration of donor derived allogeneic lymphocytes (“...immunotherapy with mononuclear cells from the marrow donor...,” last sentence of abstract). Similarly, Slavin *et al.* teaches, with respect to the experiments described therein on mice, administration of allogeneic bone marrow (C57BL/6) with tumor cells to recipient mice (Balb/c) to simulate quantitative minimal residual disease (MRD) in the context of allogeneic bone marrow transplantation (BMT). This is in very sharp contrast, however, to the instant invention which teaches administration of autologous stem cells prior to administration of allogeneic lymphocytes. It will be clear to the ordinarily skilled artisan that such transplantation of allogeneic donor derived cells prior to administration of the donor derived lymphocytes to a host, as suggested by Johnson *et al.* and Slavin *et al.*, may serve to prime the immune system of the host against donor antigens, and thereby to prime the immune system of the host to mount a particularly vigorous and rapid rejection of the donor lymphocytes. An ordinarily skilled artisan following the teachings of Johnson *et al.* or Slavin *et al.* would therefore be motivated to administer the lymphocytes to the host as rapidly as possible following transplantation of the allogeneic stem cells, and would in contrast to the Examiner's assertion not be motivated to allow any substantial hematopoietic recovery of the host

prior to administration of the allogeneic lymphocytes so as to attempt to prevent their elimination by the primed immune system of the host prior to their exertion of anti cancer activity. Moreover, Applicant wishes to point out that Johnson *et al.* describe mild GVH in non-tumor-bearing animals having undergone administration of donor derived allogeneic lymphocytes following administration of donor derived allogeneic stem cells (page 332, column 1 last paragraph to column 2 first paragraph) following lethal irradiation (materials and methods). However, the ordinarily skilled artisan would assume that in tumor bearing animals, that any GVL activity by the administered allogeneic lymphocytes could lead to pro inflammatory events, such as Th1 type cytokine secretion (e.g., IL-2, IFN-gamma TNF) which would be liable to exacerbate the GVH response to significantly more severe stages than the mild responses reported.

As such, Applicant is of the very strong opinion that none of the instant claims are rendered obvious by Slavin in view of Johnson *et al.* and Slavin *et al.*

Nevertheless, in the interest of expediting prosecution of the instant application, Applicant now elects to:

(i) amend claim 20 so as to replace the limitation of administering lymphocytes in a regimen that causes a clinically mild GVH response, with the limitation of administering lymphocytes in a regimen selected so as to cause at least partial engraftment of the lymphocytes;

(ii) amend claim 20 to comprise the step of administering to the patient, in a regimen selected so as to cause minimal GVHD, a dose of stem cells derived from a stem cell donor, where said dose of stem cells is administered to the patient following administration of the lymphocytes (support for amending the claims to include the step of administering stem cells in a regimen selected so as to cause minimal GVHD following administration of the lymphocytes is provided in the specification, pages 37-38, section entitled "Patient No. 8," and page 50, paragraph entitled "Patient No. 8");

(iii) add new claims 52-55, including, respectively, the limitations of said stem cell donor being: fully HLA-matched with the patient; at least HLA-haploidentical with the patient; HLA haplotype mismatched with the patient at a single HLA locus; and a sibling of the patient, respectively. Support for adding new claims 52-55 using

the language employed therein is provided in the specification (for example, refer to second paragraph of page 11);

(iv) add new claims 56-57 depending from claim 20, including the limitations, respectively, of said stem cell donor and said lymphocyte donor being syngeneic with each other; and said lymphocyte donor being said stem cell donor;

(v) add new claim 41 depending from claim 20 including the limitation of administering lymphocytes in a regimen selected so as to cause full engraftment of the lymphocytes;

(vi) amend claim 20 so as to include the additional step of administering a dose of allogeneic stem cells in a regimen selected so as to cause minimal GVHD;

(vii) add new claim 60, depending from claim 20, including the limitation of administering lymphocytes during a period selected from the range of 90 to 124 days following said autologous stem cell transplantation;

(viii) add new claim 61, depending from claim 20, including the limitation of administering allogeneic stem cells following administration of lymphocytes;

(ix) add new claim 63, depending from claim 20, including the limitation of administering allogeneic stem cells following at least partial engraftment of previously administered lymphocytes;

(x) add new claim 40, depending from claim 20, including the limitation of the patient being in partial remission with respect to the cancer prior to administration of lymphocytes;

(xi) add new claim 42, depending from claim 20, including the limitation of administering lymphocytes in a split dose which includes a first administration of lymphocytes in a regimen selected so as to not lead to substantial engraftment thereof;

(xii) add new claim 43, depending from claim 20, including the limitation of administering lymphocytes in a dose selected from a range of about ten million cells per kilogram to about one billion cells per kilogram;

(xiii) add new claims 44-51, depending from claim 20, including the limitation of administering allogeneic stem cells in a regimen selected so as to cause specific types of minimal GVHD, as indicated; and

(xiv) add new claim 64 including the limitation of administering the lymphocytes only during a period which: starts more than about one day following

ASCT; starts about 5 weeks, 6 weeks, 7 weeks, 55 days, 8 weeks, 58 days, 10 weeks, 71 days, 11 weeks, 90 days, 14 weeks, or 20 weeks following ASCT; and/or ends about 20 weeks following ASCT (support in the specification for such time ranges is provided in Example 2, as discussed hereinabove).

Support for amending claim 20 so as to include the above described limitations using such amendment language and for adding such new claims described in (i)-(xiii) above using the language employed therein is provided in the specification, particularly in pages 37-38, section entitled "Patient No. 8".

Applicant is therefore of the very strong opinion that in light of the above comments and amendments, claim 20 of the instant application and all claims depending directly or indirectly therefrom are patentable over Slavin (1992) in view of Johnson et al. (1993) and Slavin et al. (1990).

35 U.S.C. § 112, First Paragraph, Rejections

The Examiner has rejected claim 29 under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time the application was filed, had possession of the claimed invention. The Examiner's rejections are respectfully traversed. Claim 29 has now been amended.

In particular, the Examiner contends that the state of the biological arts at the time the instant invention was made were such that no methods were available for obtaining one's one fetal tissue as would be encompassed by the instant invention, as claimed. The Examiner further contends concedes that cord blood could be retained from birth, but that this requirement is not disclosed in the specification and that it was not a common practice at the time the instant invention was made.

Applicant is in disagreement with the Examiner's contention that the state of the biological arts at the time the instant invention was made were such that no methods were available for obtaining ones own fetal tissues. Applicant is also in sharp disagreement with the Examiner's contention that the harvesting of cord blood for transplantation was an uncommon practice at the time the instant invention was made. Applicant wishes to point that, in very sharp contrast with the Examiner's contention, at the time the instant invention was made the harvesting and long term

cryopreservation of cord blood for use in transplantation, and the successful use of cord blood in transplantation for treatment of a malignant disease had been achieved and were well known to the ordinarily skilled artisan (refer, for example, to enclosed abstracts of: Harris DT., 1994; and Newton *et al.*, 1993). Nevertheless, in the interest of expediting prosecution of the instant application, Applicant has elected to amend claim 29 to include the sole limitation of the stem cells being obtained from umbilical cord blood.

In view of the above arguments and amendments, Applicant believes to have overcome the 35 U.S.C. § 112, first paragraph, rejections.

***35 U.S.C. § 101 Rejections - Claims 1 and 7-14 of
U.S. Patent No. 5,928,639***

The Examiner has rejected claims 20 and 27-34 under 35 U.S.C. § 101 as claiming the same invention as that of claims 1 and 7-14 of prior U.S. Patent No. 5,928,639 (hereinafter "reference patent"). The Examiner's rejections are respectfully traversed. Claim 20, from which claims 27-34 depend, has now been amended. New claims 52-57 have now been added.

Applicant wishes to respectfully point out that claim 1 of the reference patent teaches administration of allogeneic lymphocytes in any regimen that causes a clinically significant graft-versus-malignant cell response, hence inherently including in a regimen which also causes a GVHD response of any severity, or in a regimen which does not cause any GVH response at all. In very sharp contrast, however, claim 20 of the instant application teaches administration of allogeneic lymphocytes in a regimen which is limited to one that causes a clinically mild graft-versus-host response. As such, Applicant is of the strong opinion that, on this basis alone, the claims of the instant application clearly do differ in scope relative to those of the reference patent, in sharp contrast to the Examiner's contention that these do not. Applicant wishes to point out that such basis indicates that the claims are indeed different in scope from those of the reference patent since the instant specification clearly specifies that GVL may occur in the absence of GVHD (page 23, lines 21-22), and since prior art cited in the instant application (refer, for example, to enclosed abstract of Slavin *et al.*, 1990a) indicates that GVL and GVHD responses may be

uncoupled. As such, since the claims of the reference patent and those of the instant application differ exclusively in their being limited to distinct regimens involving induction of GVL or GVHD responses, respectively, it can be concluded that the claims of the reference patent and those of the instant application indeed clearly and significantly differ in scope.

Nevertheless, in the interest of expediting issuance of the instant application, Applicant now elects to amend claim 20, from which claims 27-34 depend, to comprise: the step of administering lymphocytes without the limitation of their being derived from peripheral blood, and without the limitation of the administration thereof being in a regimen that causes a clinically mild GVH response; and, critically, as described above in Applicants response to the 35 U.S.C. § 103(a) Rejections, to now comprise the step of administering to the patient, in a regimen selected so as to cause minimal GVHD, a dose of stem cells derived from a stem cell donor, where said dose of stem cells is administered to the patient following administration of the lymphocytes. Applicant now further concomitantly elects, as described above in Applicant's response to the 35 U.S.C. § 103(a) Rejections, to add new claims 52-57.

In view of the amendments and explanations set forth above, Applicant believes to have overcome the 35 U.S.C. § 101 rejections.

Non-statutory obviousness-type double-patenting rejections -

Claim 1 of U.S. Patent No. 5,928,639

The Examiner has rejected claim 21 under under the judicially created doctrine of obviousness-type double-patenting as being unpatentable over claim 1 of U.S. Patent No. 5,928,639. The Examiner's rejections are respectfully traversed. Claim 20, from which claim 21 depends, has now been amended. New claims 52-57 have now been added.

As described above in Applicant's response to the 35 U.S.C. § 101 Rejections, Applicant is of the very strong opinion that claim 20 and all dependents thereof, including claim 21, are indeed patentably distinct over claim 1 of U.S. Patent No. 5,928,639. Nevertheless, in the interest of expediting issuance of the instant application, Applicant has elected to amend independent claim 20, from which claim

21 depends, and to concomitantly now add new claims 52-57, as described above in Applicant's response to the 35 U.S.C. § 101 Rejections.

In view of the amendments and arguments set forth above, Applicant believes to have overcome the non-statutory obviousness-type double-patenting rejections.

In view of the above amendments and remarks it is respectfully submitted that claims 20-21, 27-34 and 39-64 are now in condition for allowance. Prompt notice of allowance is respectfully and earnestly solicited.

Respectfully submitted,



Sol Sheinbein

Registration No. 25,457

Date: January 4, 2003.

Encl.:

A three-months extension fee;

A response transmittal fee for added claims;

The following Abstracts:

- Abstract of Bassukas ID., 1992 Med Hypotheses 38:334;
- Abstract of Beatty PG., 1994. Transfus Med Rev. 8(1):45-58;
- Abstract of Buckley RH. *et al.*, Semin Hematol. 1993, 30(4 Suppl 4):92-101; discussion 102-4;
- Abstract of Dickinson AM. *et al.*, Bone Marrow Transplant. 1994 Jan;13(1):65-70;
- Abstract of Guiot HF. *et al.*, Eur J Haematol. 1987 Feb;38(2):187-96;
- Abstract of Harris DT. *et al.*, Bone Marrow Transplant. 1994 Feb;13(2):135-43;
- Abstract of Henslee-Downey PJ., Am J Pediatr Hematol Oncol. 1993 May;15:150-61;
- Abstract of Hymes *et al.*, J Am Acad Dermatol. 1985 Mar;12(3):468-74;
- Abstract of Kohler *et al.*, 1988. Cancer Immunol Immunother. 26:74-82;
- Abstract of Newton *et al.*, 1993. Exp Hematol. 1993 May;21(5):671-4;
- Abstract of Przepiorka D. *et al.*, Bone Marrow Transplant. 1995 Jun;15(6):825-8;
- Abstract of Renkonen and Hayry, Bone Marrow Transplant. 1987 Dec;2(4):333-46;
- Abstract of Rowbottom *et al.*, 1993. Bone Marrow Transplant. 12(6):635-41;
- Abstract of Sahmoud *et al.*, Anal Cell Pathol. 1993 Sep;5(5):289-97;
- Abstract of Slavin *et al.*, 1990a. Bone Marrow Transplant 6:155;

Abstract of Vogelsang GB. et al., N Engl J Med. 1985 Sep 12;313(11):645-50;
Abstract of Weisdorf *et al.*, Gastroenterology. 1983 Nov;85(5):1076-81.

Med Hypotheses. 1992 Aug;38(4):334-8.

[Related Articles, Links](#)**Is erythema toxicum neonatorum a mild self-limited acute cutaneous graft-versus-host-reaction from maternal-to-fetal lymphocyte transfer?****Bassukas ID.**

Department of Dermatology, University Erlangen-Nurnberg, Germany.

Erythema toxicum neonatorum (ETN) is a common, self-limited neonate dermatosis affecting worldwide about 50% of newborns--mostly second and later deliveries--irrespective of sex and race. Its etiology still remains obscure: some reaction of the skin of the newborn in adapting to its new environment is the favorite hypothesis to date. A suggested viral or allergic nature could not be confirmed by adequate agent or antigen isolation respectively. In this paper a hypothesis is presented that ETN is a self-limited acute cutaneous graft-versus-host reaction (GVHR) caused in the transiently immunosuppressed newborn by maternal lymphocytes transferred shortly prior to or during delivery.

Publication Types:

- Review
- Review, Tutorial

PMID: 1491634 [PubMed - indexed for MEDLINE]

Transfus Med Rev. 1994 Jan;8(1):45-58.

Related Articles, Links

The immunogenetics of bone marrow transplantation.

Beatty PG.

Bone Marrow Transplant Program, University of Utah Health Sciences Center, Salt Lake City 84132.

It is now clear that it is not necessary to use an HLA genotypically identical donor to have a successful marrow transplant. However, it is equally clear that the likelihood of complications increases with each increment in histoincompatibility. The implication is that histocompatibility testing must be of the highest possible precision to choose the optimal donor, and to predict the risk of adverse alloreactivity. Most clinicians would seriously consider transplantation from a one-locus-mismatched relative or an HLA-matched unrelated donor in virtually any situation in which transplantation from a matched sibling would be felt to be the standard of care. More thought would need to go into transplantation from a two or three locus-mismatched relative or a mismatched unrelated donor.

Publication Types:

- Review
- Review, Tutorial

PMID: 8136607 [PubMed - indexed for MEDLINE]

Semin Hematol. 1993 Oct;30(4 Suppl 4):92-101; discussion 102-4.

[Related Articles, Links](#)

Haploidentical bone marrow stem cell transplantation in human severe combined immunodeficiency.

Buckley RH, Schiff SE, Schiff RI, Roberts JL, Markert ML, Peters W, Williams LW, Ward FE.

Department of Pediatrics, Duke University Medical Center, Durham, NC 27710.

From May 1992 to March 1993, 50 infants with severe combined immunodeficiency (SCID) were given bone marrow transplants at Duke University Medical Center. None received chemotherapy for conditioning or for graft-versus-host disease (GVHD) prophylaxis. Forty-one received haploidentical parental marrow depleted of T cells by soybean lectin and sheep red blood cell resetting, and nine received HLA-identical marrow. Forty (80%) survived from 1 week to almost 11 years posttransplantation, including nine of nine (100%) HLA-identical marrow recipients and 31 of 41 haploidentical recipients. T-cell function was present within 2 weeks after transplantation of unfractionated HLA-identical marrow, but not until 3 to 4 months after T-cell-depleted haploidentical marrow stem cells. All 37 patients who are more than 4 months posttransplantation have good T-cell function, and all but one have 100% donor T cells. B-cell function developed slowly or not at all in some recipients of haploidentical marrow. Fourteen (four HLA-identical and 10 haploidentical recipients) have some donor B cells; 19 patients are receiving intravenous immune globulin (IVIG) therapy.

Publication Types:

- Review
- Review, Tutorial

PMID: 7905667 [PubMed - indexed for MEDLINE]

Bone Marrow Transplant. 1994 Jan;13(1):65-70.

[Related Articles](#), [Links](#)

Cytokine involvement in predicting clinical graft-versus-host disease in allogeneic bone marrow transplant recipients.

Dickinson AM, Sviland L, Hamilton PJ, Usher P, Taylor P, Jackson G, Dunn J, Proctor SJ.

Department of Haematology, Royal Victoria Infirmary, Newcastle-upon-Tyne, UK.

An in vitro skin explant model has been used to predict the severity of acute graft-versus-host disease (GVHD) in 34 HLA-identical bone marrow transplant recipients (correlation coefficient 0.6 $p < 0.001$). Supernatants from HLA-matched patient/donor mixed lymphocyte cultures (MLCs) were analysed for levels of tumour necrosis factor alpha (TNF alpha) and interferon-gamma (IFN gamma). High levels of both cytokines correlated with the development of GVHD grades II or above ($p < 0.05$). The supernatants were also tested for induction of class II MHC antigen expression on third party skin and results correlated with clinical outcome in 17 of 22 cases (77%) (correlation coefficient 0.65, $p < 0.001$). The results suggest that measurement of both TNF alpha and IFN gamma in HLA-matched MLC supernatants is of predictive value and that the skin explant model is a useful model for studying the aetiology of GVHD in humans.

PMID: 8019455 [PubMed - indexed for MEDLINE]

Eur J Haematol. 1987 Feb;38(2):187-96.

Related Articles, Links

Protein loss during acute graft-versus-host disease: diagnostic and clinical significance.

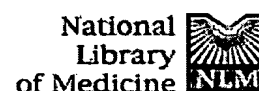
Guiot HF, Biemond J, Klasen E, Gratama JW, Kramps JA, Zwaan FE.

In 31 consecutive patients who received an allogeneic bone marrow transplantation the loss of proteins during the period at risk for acute graft-versus-host disease (aGVHD) was studied in order to determine whether the quantity of protein loss could be used for grading the severity of aGVHD. It was shown that the grade classified on the basis of the severity of skin rash, the quantity of diarrhea and the seriousness of cholestasis, correlated with serum albumin loss, intestinal plasma loss (expressed by the intestinal alpha 1-antitrypsin clearance) and the occurrence of inflammatory cells (leukocytes) in feces. The quantity of albumin lost by intestinal route accounted for only one third of the total albumin loss. To investigate whether the remaining part of it could be explained by capillary leakage elsewhere in the body, leakage of antileukoprotease from the tissue of the respiratory tract into the blood was measured. It was shown that the serum concentration of this proteinase inhibitor correlated with albumin loss. This means that capillary leakage also occurs in the lung during aGVHD. In conclusion, the loss of proteins can be used as a parameter of the severity of aGVHD once the proper diagnosis has been established. It appears that a combination of the current 'familiar' grading system and SAL yields a more objective classification system with a greater prognostic value.

Publication Types:

- Clinical Trial
- Randomized Controlled Trial

PMID: 3297772 [PubMed - indexed for MEDLINE]



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☐ 1: Bone Marrow Transplant. 1994 Feb;13(2):135-43.

Related Article

Collection, separation and cryopreservation of umbilical cord blood for use in transplantation.**Harris DT, Schumacher MJ, Rychlik S, Booth A, Acevedo A, Rubins Bard J, Boyse EA.**

Department of Microbiology & Immunology, University of Arizona, Tucson 85721.

Bone marrow transplantation (BMT) is limited by the paucity of HLA-matched donors and the frequent occurrence of graft-versus-host disease (GVHD). clinical reports have implied that the use of umbilical cord blood (UCB) may alleviate some of the problems associated with BMT. Banks of frozen UCB could make the problem of finding suitable stem cell donors easier and stem grafts would be more readily available. However, definitive experiments needed to develop optimal methods for collection, separation and storage of cryopreserved UCB for extended periods of time. We have found that several simple techniques may be utilized to collect large volumes of UCB (up to 100 ml). Also, modification of a common density gradient separation method allows recovery of large quantities of UCB mononuclear cells. Finally, we have examined the effects of prolonged frozen storage on the ability to recover and functional UCB, particularly stem/progenitor cells. It was observed that storage of UCB in liquid nitrogen for as long as 7 years had minimal effect on cell viability, cellular composition of UCB and progenitor/stem cell capacity. Thus, the establishment of UCB banks for use in transplantation appears to be a feasible approach.

PMID: 8205082 [PubMed - indexed for MEDLINE]

☐ 2: Blood Cells. 1994;20(2-3):587-96; discussion 596-600.

Related Article

Comment in:

- Blood Cells. 1994;20(2-3):627-9.

Unrelated placental blood for bone marrow reconstitution: organization of the placental blood program.**Rubinstein P, Taylor PE, Scaradavou A, Adamson JW, Migliaccio G.**

Am J Pediatr Hematol Oncol. 1993 May;15(2):150-61.

Related Articles, Links

Choosing an alternative bone marrow donor among available family members.

Henslee-Downey PJ.

Department of Medicine and Pediatrics, University of South Carolina, Columbia.

PURPOSE: Bone marrow transplantation (BMT) can be curative for patients with hematological malignancies, marrow failure syndromes, and certain metabolic disorders. However, fewer than half of the patients who could benefit have a donor who is either an HLA-matched sibling or a phenotypically similar unrelated volunteer. **PATIENTS AND METHODS:** The opportunity for allogeneic bone marrow transplantation is significantly increased by the use of partially mismatched, haploidentical related donors. The likelihood of finding a donor within the family is dependent on the acceptable degree of mismatch on the unshared chromosome. The degree of mismatch is expected to be approximately 10% for a one-antigen mismatch, 20-30% for a two-antigen mismatch, and > 95% for a three-antigen mismatch. Improvements in transplant techniques designed to enhance engraftment and to prevent the development of severe acute and chronic graft-versus-host disease have increased the feasibility of utilizing this readily available donor pool. Obviously, the ability to use a three-antigen mismatched related donor would make bone marrow transplantation available for almost every patient. **RESULTS:** Recent studies combining in vitro and in vivo immunomodulation of donor lymphocytes have resulted in consistent engraftment and control of acute graft-versus-host disease. **CONCLUSIONS:** These findings provide encouragement that there may need be no restriction on the availability of allogeneic marrow transplants as a therapeutic option for appropriate candidates.

Publication Types:

- Review
- Review, Academic

PMID: 8498640 [PubMed - indexed for MEDLINE]

J Am Acad Dermatol. 1985 Mar;12(3):468-74.

[Related Articles, Links](#)

Cutaneous graft-versus-host reaction: prognostic features seen by light microscopy.

Hymes SR, Farmer ER, Lewis PG, Tutschka PJ, Santos GW.

Acute graft-versus-host disease (GVHD) is a severe complication of bone marrow transplantation. The diagnosis may be made and its course followed by serial skin biopsies. The degree of epidermal change has been used as a guideline in grading each biopsy, but great variation may be found within each grade, especially grade 2 (basal cell vacuolization and dyskeratosis). To find a histologic parameter that is prognostic of more severe acute GVHD, we examined retrospectively the serial biopsies of 54 patients. When we studied early cutaneous graft-versus-host reaction (GVHR), represented by the grade 2 biopsies, the number of dermal and epidermal mononuclear inflammatory cells correlated positively with the probability of developing more severe acute GVHD. In addition, the patients who had more severe acute GVHD tended to have an earlier appearance of cutaneous histologic changes. None of the other histologic parameters examined in these grade 2 biopsies were found to be predictive of GVHD progression. In addition, no histopathologic parameters in these grade 2 biopsies were predictive of the subsequent development of chronic GVHD.

PMID: 3989008 [PubMed - indexed for MEDLINE]

Cancer Immunol Immunother. 1988;26(1):74-82.

Related Articles, Links

Clinical adoptive chemoimmunotherapy with allogeneic alloactivated HLA-haploidentical lymphocytes: controlled induction of graft-versus-host-reactions.**Kohler PC, Hank JA, Minkoff DZ, Sondel PM.**

Department of Human Oncology, University of Wisconsin, Madison.

A total of 13 cancer patients were treated with Adoptive Chemoimmunotherapy (ACIT) using alloactivated HLA haploidentical lymphocytes. Donor lymphocytes were activated in vitro using a pool of irradiated allogeneic lymphocytes (MLC-cells) and some further expanded by culturing in T-cell growth factor (TCGF-cells). The first 6 patients received i.v. cyclophosphamide (CPM) followed 24 h later by escalating doses of MLC-cells; then 7 days later they received an infusion of TCGF-cells. Minimal toxicity was seen. The next 7 patients received CPM (800 mg/m²) and a combined MLC and TCGF-cell infusion (total cell dose ranged from 0.79×10^{10} to 2.26×10^{10}). Of these 7 patients, 3 developed mild graft-versus-host reaction (GVHR) which resolved without treatment, and 2 patients had progressive GVHR which was arrested by methylprednisolone (2 mg/kg). Peripheral blood lymphocytes from these 2 patients, during the GVHR, had increased activated T-cells (OKT-10+ and OK-Ia+). In vitro expansion, in TCGF, of these activated T-cells enabled HLA typing to prove they were of donor origin. Only 1 clinical antitumor response was observed in the first 6 patients. The results of this study indicate that this form of ACIT can be given to patients with acceptable toxicity. Self-limited or easily controlled GVHR may be induced and primed donor cells persisting in the circulation are probably responsible. Further testing is required to determine whether the immune response induced by this form of ACIT may be therapeutically effective.

PMID: 3257904 [PubMed - indexed for MEDLINE]

Exp Hematol. 1993 May;21(5):671-4.

Related Articles, Links

Toward cord blood banking: density-separation and cryopreservation of cord blood progenitors.**Newton I, Charbord P, Schaal JP, Herve P.**

INSERM/CRTS, Besancon, France.

It has been shown that cord blood collected at birth can be used to successfully engraft a human lymphocyte antigen (HLA)-matched sibling suffering from a malignant disease. It has been further suggested that this source of cells may be used in unrelated but HLA-compatible patients. These wide indications would imply the establishment of cord blood banks comprising 10(5) or more samples. In this report we show that it is possible to fractionate and freeze cord blood samples without major loss in granulomonocytic or erythroblastic progenitors (CFU-GM and BFU-E). Density separation should be carried out using Percoll of density 1.080. Separated samples should be frozen and thawed in the presence of DNase I. This procedure should allow the storage of approximately 10 mL samples in cryotubes containing a number of CFU-GM and BFU-E sufficient to engraft a patient weighing less than 30 kg. These data provide a rationale for establishing cord blood banks.

PMID: 8513869 [PubMed - indexed for MEDLINE]

Bone Marrow Transplant. 1995 Jun;15(6):825-8.

Related Articles, Links

1994 Consensus Conference on Acute GVHD Grading.

Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J, Thomas ED.

University of Texas MD Anderson Cancer Center, Houston 77030, USA.

Grading acute graft-versus-host disease (GVHD) is usually based on quantification of rash, serum bilirubin and diarrhea. Standard criteria have been developed and used for > 20 years by most transplant centers. However, neither the standard GVHD grading system nor any of several revisions has been validated in the context of GVHD prophylaxis with cyclosporine. The 1994 Consensus Conference on Acute GVHD Grading held in Keystone in January 1994 provided an opportunity to: (1) review data regarding these standard criteria; (2) determine if there are sufficient data to revise these criteria; and (3) develop recommendations for reporting results of GVHD prevention trials. Data were provided for 8249 patients from 12 large transplant centers and 2 transplant registries. Standard GVHD grading criteria were found to distinguish different mortality risks and treatment response rates. Analysis of new data suggested that persistent nausea with histologic evidence of GVHD but no diarrhea be included as stage 1 gastrointestinal GVHD. Additional studies were recommended to evaluate heterogeneity of outcome within GVHD grades prior to making further revisions. To improve comparability between publications, reports of GVHD prevention trials should include an accurate description of the grading system used and should report actuarial rates of grades II-IV and III-IV GVHD corrected for graft failure and potential interventions for early relapse. Additional information should include indications for therapy of GVHD and response.

Publication Types:

- Consensus Development Conference
- Review

PMID: 7581076 [PubMed - indexed for MEDLINE]

Bone Marrow Transplant. 1987 Dec;2(4):333-46.

[Related Articles, Links](#)

Cellular infiltrates in the target organs associated with acute graft-versus-host disease.

Renkonen R, Hayry P.

Transplantation Laboratory, University of Helsinki, Finland.

Acute graft-versus-host disease (AGVHD) has been one of the major obstacles in successful bone marrow transplantation for many years. The histologic manifestations of AGVHD have been well defined but much less is known about the cellular infiltrates in the target organs during AGVHD, the structure of the inflammation is different in different target organs and is not necessarily similar to classical allograft rejection. This review covers, in addition to the histologic approach, the cytologic and functional approaches to the AGVHD associated with inflammatory cells.

Publication Types:

- Review
- Review, Tutorial

PMID: 3332181 [PubMed - indexed for MEDLINE]

Bone Marrow Transplant. 1993 Dec;12(6):635-41. Related Articles, Links

Monitoring cytokine production in peripheral blood during acute graft-versus-host disease following allogeneic bone marrow transplantation.

Rowbottom AW, Riches PG, Downie C, Hobbs JR.

Department of Immunology, Charing Cross and Westminster Medical School,
London, UK.

Plasma concentrations and peripheral blood cells containing cytoplasmic cytokines were monitored during the post-transplant period in 10 patients who had received allogeneic bone marrow transplants (BMT) for the correction of inherited genetic disorders. The presence of CD14-positive cells containing cytoplasmic interleukin-1 alpha and beta in the peripheral blood was indicative of acute graft-versus-host disease (GVHD). Plasma concentrations of IL-1 alpha, IL-1 beta and TNF-alpha were significantly raised in the GVHD group when compared with the uneventful days. There was, however, poor temporal correlation between the plasma concentrations and clinical manifestations of acute GVHD. Cells containing cytoplasmic IL-6 were present in the peripheral blood when patients had clinically suspected and/or microbiologically confirmed infection. The results from this study demonstrate that analysis of peripheral blood cells for cytoplasmic IL-1 alpha and IL-1 beta are better markers of acute GVHD than is monitoring plasma concentrations of these cytokines.

PMID: 8136747 [PubMed - indexed for MEDLINE]

Anal Cell Pathol. 1993 Sep;5(5):289-97.

Related Articles, Links

Towards an objective prognostic index of acute graft-versus-host disease.

Sahmoud TM, Heudes D, Irinopoulou T, Gluckman E, Nguyen Q, Brocheriou C, Devergie A, Rigaut JP, Mary JY.

INSERM, U263, Universite Paris 7, France.

Acute graft-versus-host disease (AGVHD) is one of the major complications of allogenic non-T-depleted HLA-compatible bone marrow transplantation. It is not yet possible to predict the clinical evolution of the disease at the time of its first manifestation. Twenty patients who initiated the disease with only moderate skin involvement were selected consecutively among those followed between January 1985 and December 1988 in the Bone Marrow Transplantation Unit, Saint-Louis-Hospital, Paris. A skin biopsy was performed at the onset of the AGVHD for each patient. For each biopsy, one 5-microns thick section was fixed by Bouin's solution, stained with hematoxylin and eosin and studied by image analysis at a final calibration of 7.6 pixels/microns. Ten patients did not exceed grade I (low risk group) and ten developed a more severe grade (high risk group) in the evolution of the disease. The mean and coefficients of variation, skewness and kurtosis of dimension-, form- and texture-related parameters of the nuclei of lymphocytes infiltrating the skin were investigated for their ability to discriminate between the high and low risk groups. The best discrimination was obtained using texture-related variables. An index containing 5 texture-related variables gave the maximum separation between the two groups, with a 100% correct classification. Our results represent a learning-step towards the development of a prognostic index of AGVHD.

PMID: 8217909 [PubMed - indexed for MEDLINE]

Bone Marrow Transplant. 1990 Sep;6(3):155-61.

[Related Articles, Links](#)**The graft-versus-leukemia (GVL) phenomenon: is GVL separable from GVHD?****Slavin S, Ackerstein A, Naparstek E, Or R, Weiss L.**

Department of Bone Marrow Transplantation & Cancer Immunobiology, Hadassah University Hospital, Jerusalem, Israel.

Graft-versus-leukemia (GVL) is a major component of the overall beneficial effects of allogeneic bone marrow transplantation (BMT) in the treatment of leukemia. Although several clinical trials have suggested a direct relationship between GVL effects and acute and chronic graft-versus-host disease (GVHD), it is not yet known whether GVL can be separated from GVHD. However, several investigations in murine models of human leukemia indicate that the two may be at least partially separable. Moreover, analysis of clinical data from the International Bone Marrow Transplant Registry suggest that allogeneic BMT may be more advantageous than syngeneic BMT, regardless of the GVHD. Likewise, T lymphocyte depletion is associated with an increased incidence of relapse, independently of GVHD. Recent investigations in murine leukemia suggest that GVL-like effects may be inducible following syngeneic BMT by recombinant cytokines with no overt GVHD. Taken together, current data in experimental animals and man suggest that GVL may be at least partially separable from GVHD. Hence, further understanding of effector and target cells of GVL as well as our ability to induce antitumor effector cells, especially those that are MHC nonrestricted, may lead to new approaches for potentiating anti-tumor effector mechanisms without inducing severe, clinically overt GVHD. Successful attempts in these directions may also lead to improved results following autologous BMT as a result of activation of GVL-like effects by recombinant cytokines that are capable of activating effector cells with anti-leukemic activity in vivo, such as recombinant human IL2, alpha interferon or perhaps a synergistic combination of factors.

PMID: 2252954 [PubMed - indexed for MEDLINE]

N Engl J Med. 1985 Sep 12;313(11):645-50.

[Related Articles, Links](#)**An in vitro predictive test for graft versus host disease in patients with genotypic HLA-identical bone marrow transplants.****Vogelsang GB, Hess AD, Berkman AW, Tutschka PJ, Farmer ER, Converse PJ, Santos GW.**

Acute graft versus host disease remains a major cause of morbidity and mortality in allogeneic bone marrow transplantation. To date, no clinically useful test has been reported that will predict the occurrence of graft versus host disease in genotypic HLA-identical donor-recipient pairs. We have developed a skin-explant model using donor lymphocytes that have been sensitized against recipient lymphocytes in vitro and cocultured with the recipient's skin. Histologic changes compatible with acute graft versus host disease are found in the positive explants. To date 32 patients have been tested in a prospective manner. Among the 18 recipient-donor pairs that were positive, 16 patients were found to have histologic Grade 2 or higher graft versus host disease of the skin on biopsy. Among the 14 negative pairs, only 3 patients had histologic Grade 2 or higher graft versus host disease of the skin on biopsy. Thus, the model has a sensitivity of 84 per cent and a specificity of 85 per cent, and is a significant predictor of the histologic occurrence of graft versus host disease (P less than 0.0005 by chi-square test). The test may be useful in the selection of donors for bone marrow transplantation and in the planning of prophylaxis against graft versus host disease.

PMID: 3894963 [PubMed - indexed for MEDLINE]

Gastroenterology. 1983 Nov;85(5):1076-81.

Related Articles, Links

Gastroenterology**Graft-versus-host disease of the intestine: a protein losing enteropathy characterized by fecal alpha 1-antitrypsin.****Weisdorf SA, Salati LM, Longsdorf JA, Ramsay NK, Sharp HL.**

Severe hypoproteinemia often accompanies the development of graft-versus-host disease of the intestine in allogeneic bone marrow transplant patients. To determine whether or not protein loss occurs across the intestinal mucosa in this severe diarrheal illness, we measured fecal alpha 1-antitrypsin once per week in 24-h stool specimens from 25 consecutive patients during hospitalization for bone marrow transplantation. The mean alpha 1-antitrypsin concentration and serum clearance for these patients before transplantation were below 2.6 mg/g stool and 13.0 ml/day (upper limits for normals). Values for all patients increased moderately after pretransplant conditioning. Values for patients who did not develop graft-versus-host disease of the intestine returned to baseline levels; however, those for patients with graft-versus-host disease of the intestine became markedly and persistently elevated (concentration ranged from 16.6 to 51.1 mg/g, clearance from 66.6 to 384.5 ml/day). We conclude that mucosal protein exudation contributes to the hypoproteinemia of graft-versus-host disease of the intestine and that measurement of fecal alpha 1-antitrypsin can be used as a marker for this disease.

PMID: 6352387 [PubMed - indexed for MEDLINE]